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Serial Transplantation Reveals the Stem-Cell-Like Regenerative Potential of Adult Mouse Hepatocytes

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Previous work has shown that adult mouse hepatocytes can divide at least 18 times *in vivo*. To test whether this represents the upper limit of their regenerative capacity, we performed serial transplantation of hepatocytes in the fumarylacetoacetate hydrolase deficiency murine model of liver repopulation. Hepatocytes from adult donors were serially transplanted in limiting numbers six times and resulted in complete repopulation during each cycle. This corresponds to a minimal number of 69 cell doublings or a 7.3×10^{20}-fold expansion. No evidence for abnormal liver function or altered hepatic architecture was found in repopulated animals. We conclude that a fraction of adult mouse hepatocytes have growth potential similar to that of hematopoietic stem cells. ***(Am J Pathol 1997, 151:1273–1280)***

Stem cells play a crucial role in the physiological turnover and regenerative responses of many tissues in adult life. In inherited diseases, the goal of somatic gene therapy is to produce a permanent correction of the genetic defect underlying the pathophysiology of the disorder. For the therapeutic effect to be long lasting it may be necessary to transduce the cells responsible for tissue maintenance and regeneration. This is best illustrated by gene therapy for hematopoietic disorders in which the pluripotent stem cell has to be corrected to achieve long-term efficacy.

The liver is an important target organ for gene therapy, particularly of inherited metabolic diseases,[1] but the stem cell biology of the liver is currently not completely understood. In adult mammals, the liver is a relatively quiescent organ with a slow cell turnover.[2] However, even the adult organ retains a remarkable potential for regeneration after hepatic injury. In rodents, the liver will re-acquire its original weight within only a few days after surgical removal of up to three-fourths of the entire organ. It has been well documented that virtually all hepatocytes participate equally in this post-hepatectomy type of regeneration. In contrast, a common progenitor (hepatoblast) gives rise to both bile duct epithelial cells and differentiated hepatocytes during embryonic development.[3] This same cell may also be responsible for some forms of liver regeneration later in life.[4,5] Thus, current evidence suggests the existence of two basic types of liver regeneration. The progenitor (stem cell) dependent regeneration may be used whenever parenchymal hepatocytes are severely damaged and unable to regenerate efficiently.

Many basic questions about liver regeneration remain unanswered, because an appropriate *in vivo* assay to determine the regenerative and lineage capabilities of liver cells did not exist. Recently, however, two transgenic mouse models for clonal liver repopulation have been described.[6–8] In albumin-urokinase transgenic mice, the hepatocellular expression of this protease is toxic.[7] In mice deficient for the tyrosine catabolic enzyme fumarylacetoacetate hydrolase (FAH), accumulation of the toxic intermediate fumarylacetoacetate damages the cell.[6,9] In both systems, transplanted wild-type hepatocytes can competitively repopulate the diseased organ.[8,10] These studies have shown that some adult parenchymal hepatocytes have the ability to divide 12 to 18 times and give rise to fully functional progeny. Serial partial hepatectomies performed in rats also have shown hepatocytes to be capable of at least 18 cell doublings.[11] However, the upper limit of the regenerative capacity of hepatocytes remained unknown.

To determine the true regenerative potential of repopulating liver cells, we performed serial transplantation of a limiting number of unfractionated adult parenchymal hepatocytes in FAH-deficient mice. Our results document that such cells can divide at least 69 times without loss of function. This stem-cell-like ability to replicate has important implications for liver cell biology, hepatocellular transplantation therapy, and liver gene therapy.

Materials and Methods

Mouse Strains and Transplantation Procedures

We used an animal model of hereditary tyrosinemia type 1, the $FAH^{\Delta exon\ 5}$ strain of mice, which has previously

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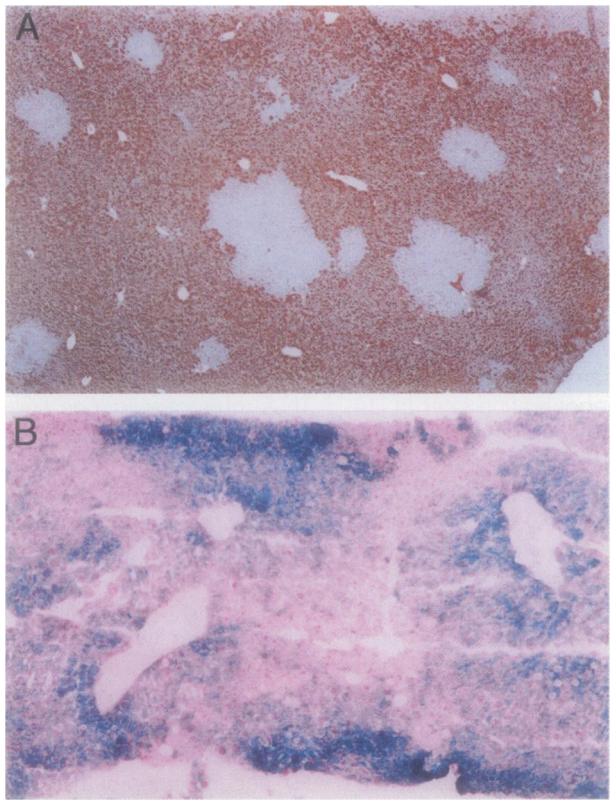

Figure 1. Histology of repopulated liver. **a:** Repopulation of FAH^- mutant female mouse liver with 10^5 FAH^+ adult male hepatocytes during the sixth round of serial transplantation. FAH expression was found by immunohistochemistry in ~80% of hepatocytes. Magnification, ×125. **b:** β-Galactosidase staining of a frozen section of mutant liver repopulated with donor hepatocytes from ROSA-26 mice during the fourth round of serial transplantation. Magnification, ×125. The presence of blue staining shows that the repopulation is caused by transplanted donor cells, not revertant endogenous hepatocytes.

been described by this laboratory.[6] All breeders and all mutant animals were treated with 2(2-nitro-4-trifluoromethylbenzoyl)-1,3 cyclohexane dione (NTBC)-containing drinking water at a concentration of 7.5 mg/L (provided by S. Lindstedt, Gøtheborg, Sweden). NTBC is a potent inhibitor of the 4-OH phenylpyruvate dioxygenase, the second enzyme of tyrosine catabolism.[12] Treatment with this drug prevents liver failure in FAH mutant mice and is necessary for the animals to survive and breed.[13] For genotyping, polymerase chain reaction was carried out with a 3′ primer on 200 ng of tail-cut DNA as previously described.[6]

All transplantation experiments were performed with congenic mice of the 129Sv background. Rosa-26 transgenic mice on the 129Sv background were provided by P. Soriano.[14] This mouse strain contains a transgene, which leads to ubiquitous expression of *Escherichia coli* β-galactosidase (lacZ) in all cell types. The lacZ expression of transplanted cells can be tracked with a histochemical reaction. Animal care and experiments were all in accordance with the Guidelines of the Department of Animal Care at Oregon Health Sciences University. Parenchymal hepatocytes were isolated from congenic male wild-type animals by a two-step collagenase perfusion.[15] Cell number and viability were determined by trypan blue exclusion in a hemocytometer. The appropriate number of donor cells were resuspended in 100 μl of Dulbecco's minimal essential media (Gibco BRL, Gaithersburg, MD) with 15% fetal calf serum and injected intrasplenically[16] into mutant female recipient animals. All mutant mice were kept on NTBC until the time of transplantation. NTBC was discontinued 2 days after the last therapeutic intervention to permit positive selection. The weight of experimental animals was measured weekly.

Table 1. Liver Function Tests in Repopulated Mice

Mouse	Alanine aminotransferase (U/L)	Conjugated bilirubin (mg/dl)	Transplant round	Minimal expansion	Minimal number of cell doublings	Probable number of cell doublings
R51	183	0.4	3	2.7×10^7	25	32
R06	50	0.1	3	2.7×10^7	25	32
R18	118	0.6	3	2.7×10^7	25	32
O88	204	0.1	3	2.7×10^8	28	35
O86	31	0.1	3	2.7×10^9	31	38
R45	86	0.1	3	2.7×10^9	31	38
701	192	0.1	3	2.7×10^{10}	35	42
756	140	0.1	3	2.7×10^{10}	35	42
R67	41	0.1	4	8.1×10^{10}	37	48
R33	47	0.1	4	8.1×10^{11}	40	51
921	55	0.1	4	8.1×10^{11}	40	51
923	161	0.1	4	8.1×10^{11}	40	51
V73	105	0.1	5	1.2×10^{12}	40	51
V76	17	0.1	5	2.4×10^{12}	41	55
V81	32	0	5	2.4×10^{12}	41	55
1316	28	0	6	7.3×10^{19}	66	82
1319	54	0.1	6	7.3×10^{19}	66	82
1359	16	0	6	7.3×10^{19}	66	82
1315	117	0	6	7.3×10^{20}	69	86
1360	36	0	6	7.3×10^{20}	69	86
1358	25	0	6	7.3×10^{20}	69	86
Control	30–90	<0.2				
Mutant	259–653	4.5–10.3				

Liver function tests (mean ± 2 SD) for FAH-deficient mice after NTBC withdrawal and for control littermates are given according to Ref. 13. The minimal number of cell doublings and expansion was calculated assuming equal repopulation by all transplanted donor cells. For the probable number, we used the more realistic assumption that, at most, 15% of donor cells participated.

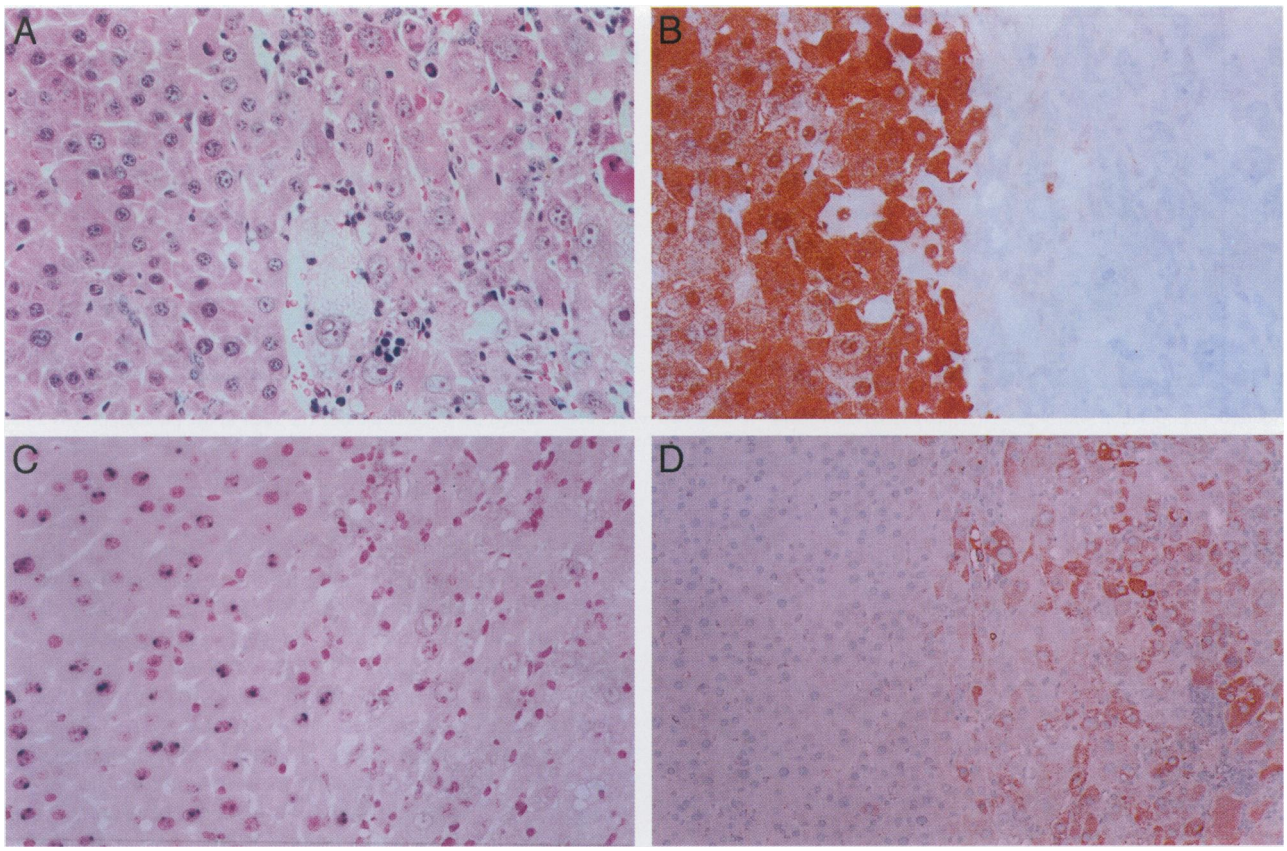

Figure 2. Serial sections of repopulated liver. Repopulation of FAH^- liver with 10^3 FAH^+ adult male hepatocytes. The photographs are focused on a field with normal donor hepatocytes on the left and residual host liver on the right. Magnification, ×400. The repopulating cells appear histologically normal (**a**), stain positive for FAH (**b**), and are Y chromosome positive (**c**). In contrast, the FAH-negative, Y-chromosome-negative hepatocytes at right show nuclear pleomorphism, necrosis, and infiltration by inflammatory cells. Host hepatocytes contained abundant α-fetoprotein (**d**), indicating their preneoplastic transformation, whereas the healthy donor cells did not.

Biochemical Analysis

Samples from animals were obtained as follows. Animals were sacrificed by decapitation and blood collected by dabbing the wound onto Parafilm. For anticoagulation, the blood was immediately mixed with 10 μl of sodium/heparin using a Pipetman. The red blood cells were removed by a brief centrifugation, and the plasma was frozen at −80°C. Twenty microliters of plasma was mixed with 80 μl of a solution of 7% bovine serum albumin and assayed for alanine aminotransferase and bilirubin levels with a Kodak Ektachem 700 chemistry analyzer. Plasma amino acids were quantitated on a Beckman 6300 automated amino acid analyzer.[17]

Northern Blots

Five micrograms of total cellular RNA was probed with the cDNAs for albumin, phosphoenolpyruvate carboxykinase, and tyrosine aminotransferase.[18]

Histology and Immune Histology

Liver tissues fixed in 10% phosphate-buffered formalin, pH 7.4, were dehydrated in 100% ethanol and embedded in paraffin wax at 58°C. Four-micron sections were rehydrated and stained with hematoxylin and eosin (H&E) and with a polyclonal rabbit antibody to rat FAH[19,20] or glutamine synthetase.[21] The FAH antibody was diluted in phosphate-buffered saline (PBS), pH 7.4, and applied at concentrations of 1:300,000 at 37°C for 30 minutes. The glutamine synthetase antibody was used at a dilution of 1:10,000. Rabbit antibody to mouse α-fetoprotein was obtained from ICN (Isle, IL; catalog item 64–561) and used at a dilution of 1:10,000. Endogenous peroxidase activity was blocked with 3% H_2O_2 and methanol. Avidin and biotin pretreatment was used to prevent endogenous staining. The secondary antibody was biotinylated goat anti-rabbit IgG used at 1:250 dilution (Vector Laboratories, Burlingame, CA; BA-1000). Color development was performed with the aminoethylcarbazole detection kit from Ventana Medical Systems (Tucson, AZ; catalog item 250–020).

β-Galactosidase histochemistry in frozen sections was performed as previously described.[22] The 8- to 10-μm-thick sections of OCT-embedded liver were fixed with 1.25% glutaraldehyde in ice-cold PBS for 10 minutes and stained overnight.

For detection of male cells, *in situ* hybridization was performed with a digoxigenin-labeled high-copy-number Y-chromosome-specific repeat DNA probe as previously described.[23]

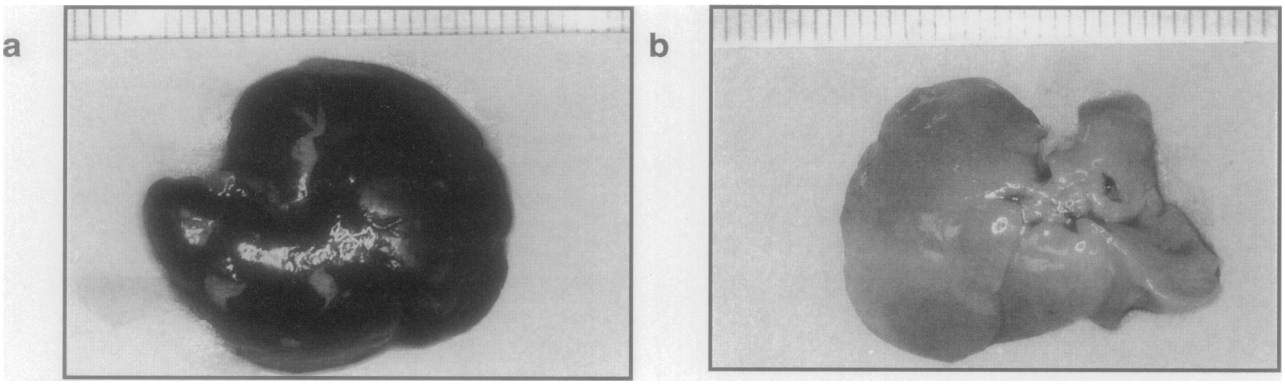

Figure 3. Repopulation with ROSA-26 hepatocytes. Macroscopic view of two whole livers stained for β-galactosidase. The ruler in the photograph has 1-mm divisions. **a:** FAH mutant liver transplanted with 10^4 ROSA 26 hepatocytes in the sixth round of serial transplantation. Repopulation with blue cells was >90% complete, whereas no blue staining was seen in the wild-type control (**b**).

Results

Serial Transplantation of Liver Cells

Our previous work has shown that 10,000 adult wild-type hepatocytes transplanted by intrasplenic injection are sufficient for complete repopulation of an FAH mutant liver.[10] Therefore, we isolated parenchymal hepatocytes from 8-week-old male congenic donors and transplanted 10,000 cells into 6-week-old mutant female recipients. Other donors were female heterozygotes for the Rosa-26 transgene[14] and expressed *E. coli* β-galactosidase in their hepatocytes. To induce liver regeneration, recipient mice were taken off the protective drug NTBC,[12,13] and selection for the wild-type cells was allowed to proceed for 8 weeks. The repopulated livers of mutant mice were then perfused with collagenase, and hepatocytes were isolated and again transplanted into fresh $FAH^{\Delta exon5}$ mutant female recipients. In this and all subsequent rounds of transplantation at least two recipients each received 10^3, 10^4, 10^5, and 10^6 donor cells via intrasplenic injection. After 8 weeks of *in vivo* selection, another round of transplantation was performed, using the animals repopulated with 10^4 or 10^5 cells as donors. In each round of transplantation, some animals were harvested for functional and histological analysis and not used for further transplantation. Surviving and repopulated mutant recipients were found with each round of transplantation. Here we report our results up to and including the sixth round. Importantly, however, the upper limit of regenerative capacity was not yet exhausted after six rounds of expansion. Animals that have received a seventh limiting transplantation are currently undergoing selection.

The degree of repopulation achieved was measured by two criteria. First, quantitative Southern blot analysis[10] was performed on DNA from repopulated livers to measure the contribution of wild-type donor cells (data not shown). Second, immune histology with an FAH antibody[10] was used to estimate the proportion of FAH-positive cells in tissue sections (Figure 1a). By both methods, we were able to demonstrate 70 to 95% overall repopulation in surviving animals, which had received 10,000 donor cells. Cell counts revealed that the livers of 2- to 3-month-old adult 129Sv mice contain 3×10^7 to 8×10^7 hepatocytes. Thus, the transplanted donor cells had to expand at least 3000-fold ($3 \times 10^7/10{,}000$) on average with each round of transplantation to achieve the observed repopulation. This figure makes the assumption that every transplanted cell migrated from the spleen to the liver and participated in the repopulation. Therefore, the minimal number of cell doublings that occurred during six rounds of repopulation can be calculated as the $\log_2$ of 3000^6, or 69.3. However, from our own work[10] and several published observations,[8,16] it can be estimated that, at most, 15% of intrasplenically delivered donor hepatocytes migrate to the liver via the portal circulation within 24 hours. If all of these participated in repopulation, this would represent an approximately 20,000-fold expansion per round of transplantation. Under this assumption, at least 85.7 cell doublings would be required for the observed six serial repopulations. Table 1 describes the round of transplantation and minimal (all transplanted cells repopulate) and probable (15% of cells repopulate) number of cell doublings in individual repopulated mice.

Repopulating Hepatocytes Are of Donor Origin

In the albumin-urokinase model of liver repopulation, somatic loss of the hepatotoxic transgene is a frequent event.[7,8,24] To prove that such a phenomenon did not account for the apparent repopulation in our experiments, we performed Southern blots with a Y-chromosome-specific probe on DNA from repopulated livers. In all cases, a high percentage (>50%) of male DNA was detected in repopulated livers (data not shown). In addition, *in situ* hybridization for Y chromosome sequences was performed.[23] FAH-positive cells were Y chromosome positive and did not show the necroinflammation and dysplasia seen in the Y-chromosome-negative, FAH-negative recipient hepatocytes (Figure 2, a–d). Because all recipients in our experiments were female mutant mice, the presence of male DNA conclusively ruled out somatic reversion.

Table 2. Plasma Amino Acid Levels in Repopulated Mice

Mouse	Tyr (μmol/L)	Phe (μmol/L)	Leu (μmol/L)	Gly (μmol/L)	Arg (μmol/L)	Met (μmol/L)	Transplant round	Minimal expansion	Minimal number of cell doublings	Probable number of cell doublings
Q52	66	65	140	204	167	77	3	2.7×10^6	21	30
Q53	65	81	159	263	199	78	3	2.7×10^6	21	30
Q59	84	67	96	209	131	62	3	2.7×10^7	25	33
R67	48	56	136	180	86	57	4	8.1×10^{10}	37	48
R33	68	69	144	209	106	49	4	8.1×10^{11}	40	51
V22	81	56	105	250	57	66	5	2.4×10^{18}	61	75
V84	77	64	149	257	49	77	5	2.4×10^{18}	61	75
Control	51 ± 26	44 ± 30	114 ± 56	238 ± 132	104 ± 28	44 ± 30				
Mutant	926 ± 386	156 ± 154	212 ± 198	713 ± 240	288 ± 330	200 ± 212				

Plasma amino acid concentrations for FAH-deficient mice after NTBC withdrawal and for control littermates are given according to Ref. 13.

Additionally, β-galactosidase staining was used in those serial transplants that used ROSA-26 mice as donors (Figure 3).[14] Repopulating FAH-positive hepatocytes were also β-galactosidase positive in all cases, indicating absence of somatic reversion (Figures 1b and 2).

Functional Capacity of Serially Repopulating Hepatocytes

Multiple assays were used to demonstrate the functional capacity of repopulating hepatocytes. Plasma levels of alanine aminotransferase and conjugated bilirubin were measured as parameters of hepatocyte integrity and liver function. Table 1 shows the results of these tests in correlation to the calculated number of cell doublings. Although not all repopulated mice had completely normal parameters, >60% of animals in each cohort did. The mild hepatic dysfunction in some mice was likely due to the presence of remaining FAH-deficient tissue. Repopulation of >85% is required for complete normalization of plasma alanine aminotransferase and bilirubin levels. Plasma amino acid levels were also measured quantitatively in several animals. To have normal levels of plasma amino acids, multiple hepatic synthetic and catabolic pathways need to be functional. As shown in Table 2, plasma amino acid levels were normal in all repopulated mice tested.

Northern blots were performed on several animals to determine the levels of some liver-specific mRNAs that are perturbed in FAH-deficient hepatocytes.[6,25] Normal mRNA levels of tyrosine aminotransferase and phosphoenolpyruvate carboxykinase were found in repopulated mice, whereas mutant mice had severely reduced levels as expected (Figure 4).

Histological examination using light microscopy showed normal morphology of repopulating hepatocytes (Figure 5a). The lobular organization of the livers was also normal. *In situ* hybridization for Y-chromosome-specific repeats revealed positive signal in hepatocytes but not endothelial cells, Kupffer cells, or biliary epithelium (Figure 5b).

To further verify the functional integrity and anatomical reconstitution of repopulated livers, immune histology with the hepatocyte-specific marker glutamine synthetase was performed.[21] This enzyme is normally expressed exclusively in the hepatoctyes immediately adjacent to terminal hepatic veins.[21] In the reconstituted livers, this distribution was identical to normal control livers (Figure 5c).

Lack of Malignant Transformation in Repopulating Cells

Because of the high number of mitosis the repopulating cells had undergone, we wanted to rule out a transformed growth phenotype. Three mice each from the third and fourth round of serial transplantation were observed for 5 months and then sacrificed. The liver weights in these animals were in the normal control range, indicating normal growth control (data not shown). Figure 2d shows that repopulating hepatocytes also did not express α-fetoprotein, a marker for preneoplastic transformation.

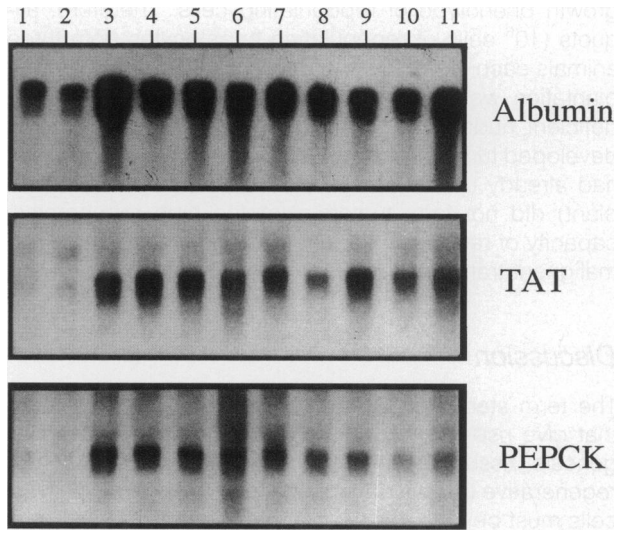

Figure 4. Northern blots of liver-specific mRNAs. TAT, tyrosine amino transferase; PEPCK, phosphoenolpyruvate carboxykinase. **Lanes 1** and **2**, FAH mutant off NTBC; **lane 3**, wild-type 2 weeks old; **lane 4** wild-type 6 months old; **lanes 5** to **7**, FAH mutant liver repopulated during the third round of serial transplantation with donor cells that have undergone >26 cell doublings; **lanes 8** and **9**, fourth round transplants with >38 cell doublings; **lanes 10** and **11**, fifth round transplants with >50 cell doublings. The TAT and PEPCK mRNA levels of repopulated mutant liver were corrected to wild-type levels.

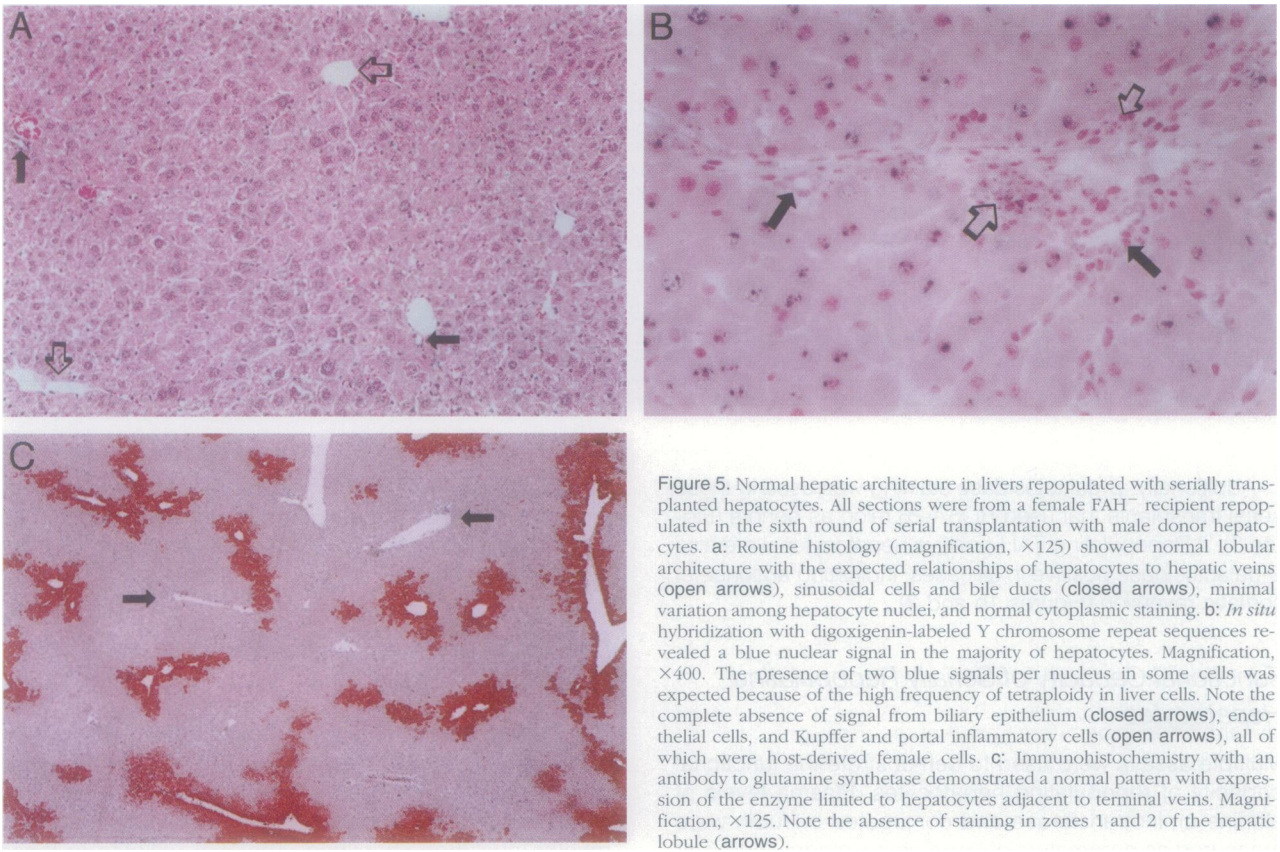

Figure 5. Normal hepatic architecture in livers repopulated with serially transplanted hepatocytes. All sections were from a female FAH$^-$ recipient repopulated in the sixth round of serial transplantation with male donor hepatocytes. a: Routine histology (magnification, ×125) showed normal lobular architecture with the expected relationships of hepatocytes to hepatic veins (open arrows), sinusoidal cells and bile ducts (closed arrows), minimal variation among hepatocyte nuclei, and normal cytoplasmic staining. b: *In situ* hybridization with digoxigenin-labeled Y chromosome repeat sequences revealed a blue nuclear signal in the majority of hepatocytes. Magnification, ×400. The presence of two blue signals per nucleus in some cells was expected because of the high frequency of tetraploidy in liver cells. Note the complete absence of signal from biliary epithelium (closed arrows), endothelial cells, and Kupffer and portal inflammatory cells (open arrows), all of which were host-derived female cells. c: Immunohistochemistry with an antibody to glutamine synthetase demonstrated a normal pattern with expression of the enzyme limited to hepatocytes adjacent to terminal veins. Magnification, ×125. Note the absence of staining in zones 1 and 2 of the hepatic lobule (arrows).

It is known that the microenvironment of the mouse liver can suppress the tumorigenic growth of transformed cells,[26] and thus the assays described above did not completely rule out the possibility of a transformed growth phenotype of repopulating cells. Therefore, aliquots (10^6 cells of repopulating hepatocytes from three animals each of the fourth and fifth rounds of serial transplantation were injected intraperitoneally into immune-deficient nude and SCID mice. None of the recipients developed tumors (data not shown). Thus, even cells that had already undergone 55 doublings (fifth-round transplant) did not form tumors, and the high regenerative capacity of repopulating cells was not explained by premalignant transformation.

Discussion

The term stem cell is used to describe precursor cells that give rise to differentiated offspring during embryogenesis, tissue regeneration, or turnover. To replenish regenerative tissues during the life of the organism, stem cells must be able to undergo many cell divisions. Some stem cells, such as the hematopoietic stem cell, give rise to multiple different cell lineages among their offspring. In embryogenesis, hepatoblasts give rise to both hepatocytes and bile duct epithelial cells. Thus, adult liver stem cells might also be expected to produce both lineages. Oval cells posses this bi-lineage capacity and for this reason have been considered to be candidates to represent liver stem cells.[27,28] However, some authors proposed that hepatocytes themselves constitute a single lineage or unipotential stem cell system.[29] In this view, all adult hepatocytes are more or less equally clonogenic, especially under the experimental conditions of partial hepatectomy.[30] Others have held that a subpopulation of progenitor cells (such as oval cells) is responsible for hepatic regeneration in specific experimental settings, especially chemical injury.[2,5,29] These two views are not necessarily mutually exclusive but could both be correct depending on the kind of injury that leads to liver regeneration. Progenitor-dependent regeneration may be used only in situations when the capacity for cell division is impaired in differentiated hepatocytes.

The hypothesis that a progenitor cell population can be responsible for hepatic regeneration predicts their very high capacity for cell division, because only a few cells would effect regeneration of the entire organ. Until now the upper limit of the mitotic capacity of liver cells has been unknown. Previous work has documented a minimal number of 18 cell doublings.[8,10,11] Here we show that the adult mouse liver contains transplantable cells that have regenerative capacity similar to that of the hematopoietic stem cell. The number of cell doublings required for successful liver repopulation during six rounds of serial transplantation is at least 69. This number likely represents a considerable underestimate of the true replicative capacity of repopulating cells because it assumes that every single transplanted cell equally participates in re-

population. Previous work by us and others indicated that, at most, 15% of intrasplenically transplanted hepatocytes migrate to the liver within 24 hours after injection.[8,10,16] If all of these 15% equally participate in repopulation, a minimum of 86 cell doublings would be required to explain our observations. This number would be even higher if the fraction of repopulating hepatocytes is more rare than the 15% that migrate to the liver. Thus, although our data clearly document the existence of highly replicative adult liver cells, it currently remains unclear what percentage of hepatocytes have this capacity. In each round of serial transplantation, some recipients were repopulated by 10,000 donor cells, indicating that the highly regenerative cells represent at least 0.01% of hepatocytes. However, it is also possible that a substantial percentage or even all adult hepatocytes have this remarkable ability. We think that the term regenerative transplantable hepatocyte (RTH) would be an appropriate designation for this putative subpopulation. Retroviral marking studies and cell sorting experiments will be required to accurately determine the number of RTHs in adult liver.

Under the experimental conditions used for this report, there was no evidence that RTHs gave rise to any other hepatic cell lineage, including bile duct epithelium, and thus their behavior differs from that reported for oval cells. The multilineage capacity of RTHs will have to be further explored by forcing proliferation of cell types other than hepatocytes in repopulating livers.

In the current study, the upper limit of cell doublings that RTHs are capable of was not yet reached. A seventh round of transplantation is currently underway, and the experiment will continue until an upper limit becomes apparent. (Since this paper was written, several mice from the seventh round of serial transplantation have survived the full selection period, indicating a new minimal estimate of 77.5 population doublings.) However, the number of cell divisions already achieved is of the same order of magnitude as in hematopoietic stem cells, and even these have been shown to lose their repopulative capacity after four to six rounds of serial transplantation.[31,32] Most populations of primary cells have an upper limit to the number of cell divisions (the Hayflick limit) they can undergo before senescence occurs. Primary murine cells are limited to 15 to 20 doublings.[33] Thus, the regenerative capacity of murine RTHs clearly exceeds the Hayflick limit for other primary cells.

An important feature of the RTHs described here is their ability to remain fully functional as hepatocytes even after transplantation and multiple rounds of expansion. After reaching the parenchyma, they divide, replace the existing hepatocytes without mass effect, and then establish the lobular organization and contact with bile ducts that are required for normal liver function. This homing ability again is similar to that of hematopoietic stem cells. Furthermore, malignant transformation of RTHs was not observed despite the high number of replication cycles they underwent. These properties make them attractive for potential use in the therapy of liver diseases by gene therapy and cellular transplantation. RTHs are the obvious choice for these treatment strategies because of their great capacity for cell division and potential for expansion *in vivo*. Based on the observation of functionally normal revertant nodules in human patients with hereditary tyrosinemia, it appears highly likely that similar cells exist in human liver.[34]

The liver repopulation and serial transplantation model described here is analogous to bone marrow repopulation studies performed in the w mice or lethally irradiated hosts years ago.[35] This system eventually led to the identification of hematopoietic stem cells and methods to purify them for therapeutic purposes. Similar experiments can now be performed in the liver and, we hope, will provide comparable results.

Acknowledgments

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